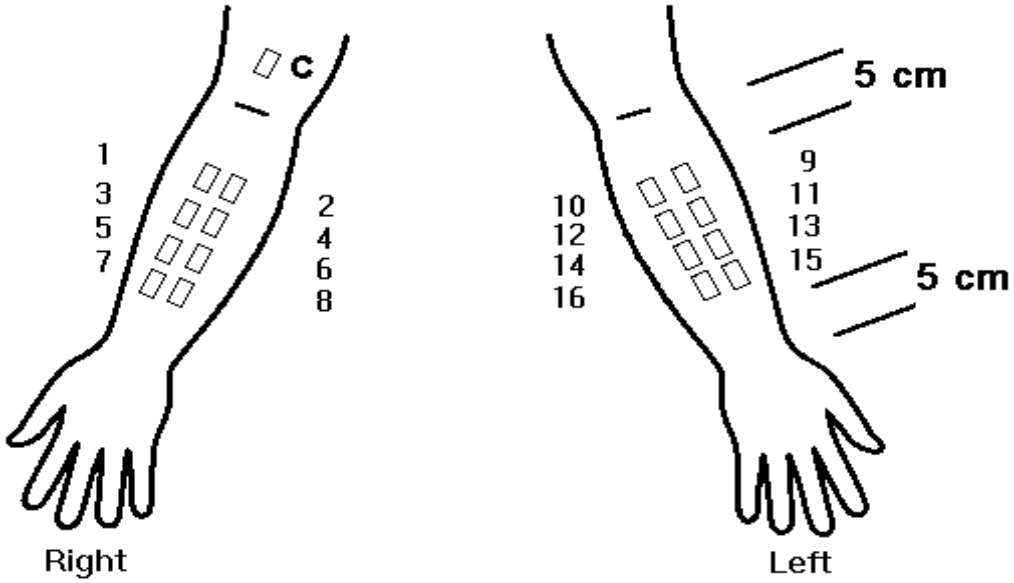
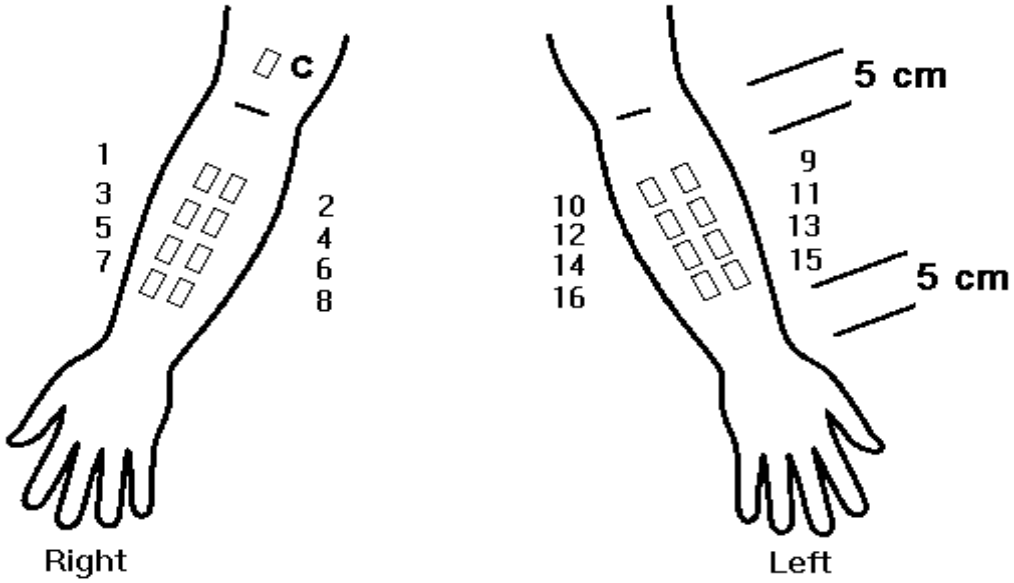
STUDY #1

Avita Gel 0.025% vs Retin-A Gel 0.025%

(36 Subjects, 14 F, 22 M; 15 C, 15 H, 4 AA, 2 AS; 32.2 yrs)



1) 0.02 mL applied to 4 cm^2 using Eppendorf Repeat Pipetter and evenly spread over the entire demarcated area with a smooth glass rod
2) Forearms covered with non-occlusive aluminum screen guard, and a cotton sleeve over the guard to minimize light exposure (absorption phase)
3) Absorption and elimination phases randomized to R or L arms
4) Products randomized between medial/lateral and proximal/distal sites



1) Absorption Phase: 0.5, 1, 2, 4 hours
2) Elimination Phase: 8, 12, 24, 48 hours
3) At each time in Absorption Phase, sites are blotted 3 times with Kimwipes, wiped once with dry cotton-tipped swab, then stripped 22 times with Transpore Tape
4) At 4 hours all sites on Elimination arm blotted with KimWipes, swabbed, and tape stripped twice. At subsequent times, 20 tape strips taken
5) Strips 1-2: Discarded
   Strips 3-12: Pooled, extracted, concentrated (8 mL to 0.1 mL), analyzed by HPLC
5) Strips 13-22: Pooled, extracted, concentrated (8 mL to 0.1 mL), analyzed by HPLC
6) Pooled tape strips extracted 48 hours in acetonitrile
7) All procedures conducted under dim yellow light

Figure 1: Summary Results: By Strip Set

Mean Tretinoin (µg) recovered from 36 subjects

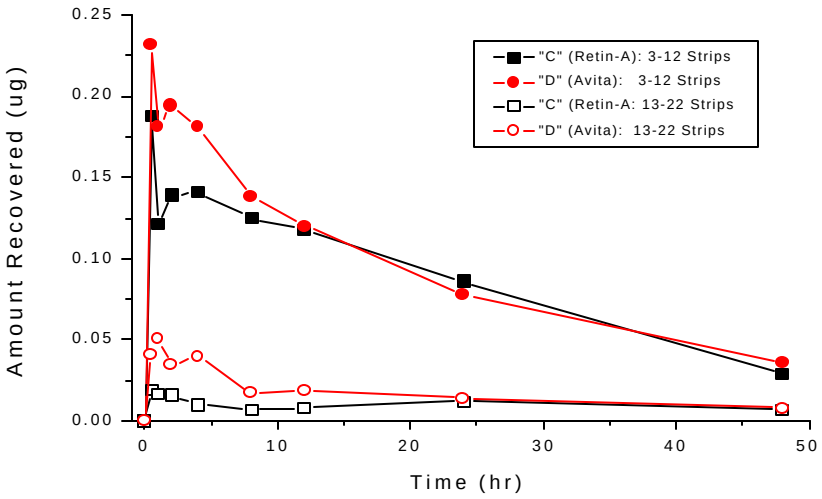
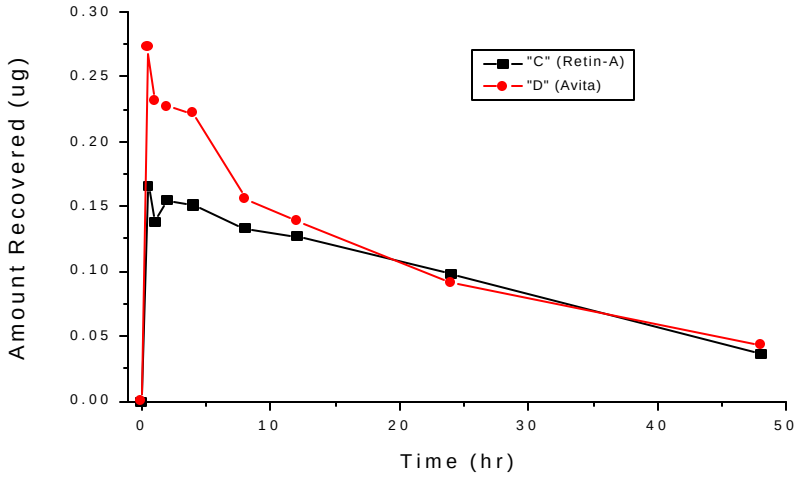


Figure 2: Summary Results: All Strips

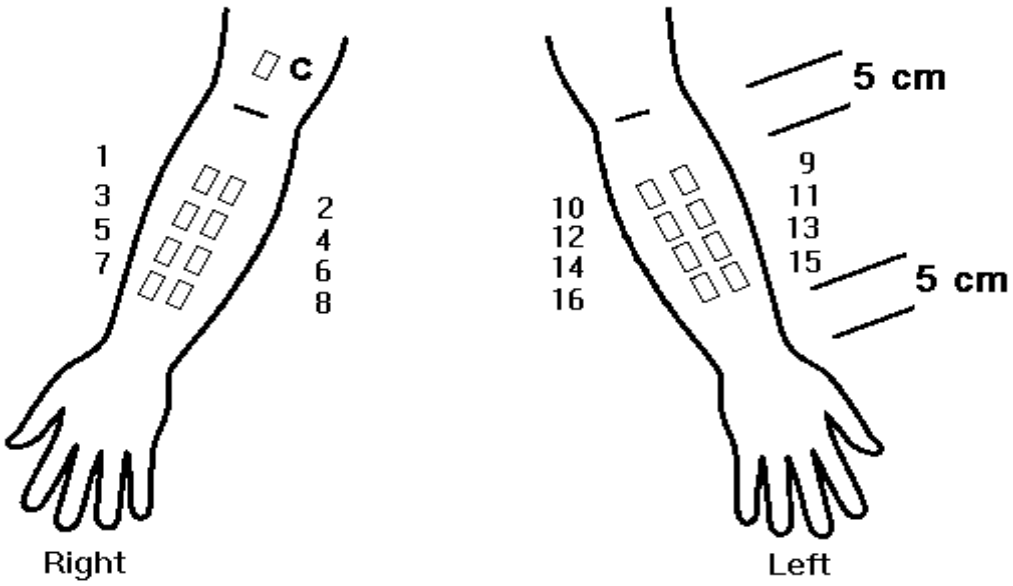
Mean Retinoic acid (µg) recovered from 36 subjects



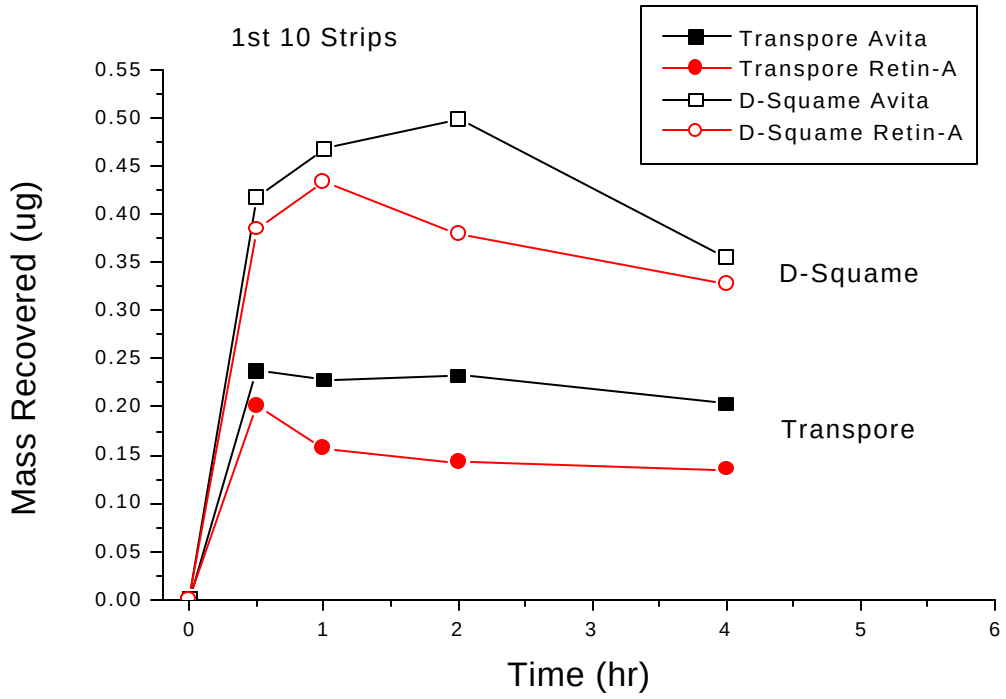
STUDY #2

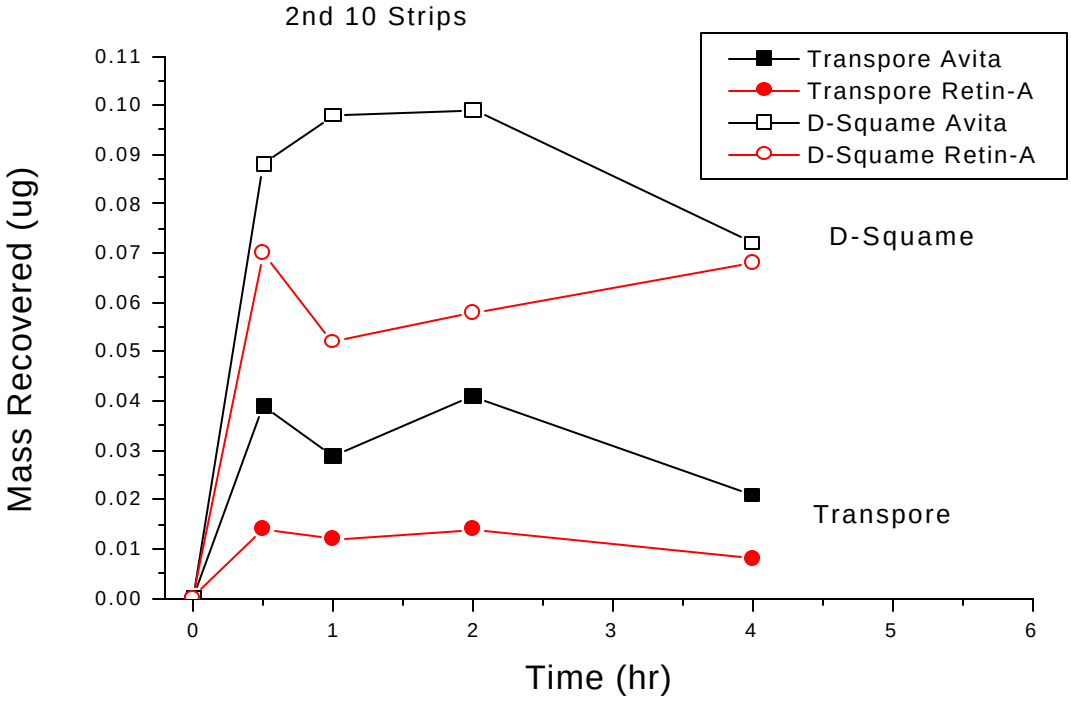
Avita Gel 0.025% vs Retin-A Gel 0.025%

(18 Subjects, 14 F, 4 M; 9 C, 4 AA, 2 H, 1 AS, 2 Un; 43.3 yrs)



1) Absorption Phase Only: 0.5, 1, 2, 4 hours
2) One arm stripped with D-Squame, other arm with Transpore
3) All procedures were the same as those used in Study #1.


1st 10 Strips
Transpore Avita
Transpore Retin-A
D-Squame Avita
D-Squame Retin-A
D-Squame
Transpore
Mass Recovered (ug)
Time (hr)
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
6


2nd 10 Strips
Transpore Avita
Transpore Retin-A
D-Squame Avita
D-Squame Retin-A
D-Squame
Transpore
Mass Recovered (ug)
Time (hr)
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6

BACKGROUND

A number of well accepted techniques are available to confirm the bioequivalence of topical generic products, and this presentation will illustrate the use of two such techniques, one kinetic-based and the other dynamic-based, that can be used with tretinoin products:

1) human cadaver skin assay (*in vitro*)
2) transepidermal water loss assay (*in vivo*)

OBJECTIVES

1. To determine the bioequivalence of two concentrations (0.01%, 0.025%) of generic tretinoin gel (Spear Pharmaceuticals), to the innovator products (Retin-A Gel®).
2. To demonstrate the concordance between two model systems that can be used to establish the bioequivalence of tretinoin products:
    (a) the human cadaver skin assay,
    (b) the transepidemal water loss assay.

METHODS

1. HUMAN CADAVER SKIN ASSAY

Dermatomed skin from eight donors was mounted on 0.8 cm^2 Franz cells with the dermal surface bathed by isotonic saline containing 0.5% Volpo 20 and maintained at 37EC. The epidermal surface was exposed to ambient laboratory conditions and dosed with 6.25 FL per cm^2 of each of the four active gel products. The dermal receptor solution was sampled at 4, 8, 12, 24, 30, 36, and 48 hours and analyzed for radioactive content. Following the 48 hour sample, the skin surface was washed twice with isopropanol to remove unabsorbed drug, then separated into epidermis and dermis, and all specimens analyzed separately for radioactive content. The primary variables used to determine bioequivalence were: (a) area under the absorption curve (AUC), (b) maximum flux (F_{max}), and (c) time of maximum flux (T_{max}). All analyses were based on log transformed data.

METHODS

2. TRANSEPIDERMAL WATER LOSS ASSAY

Daily application of test products (0.02 mL) were made for 20 days to 5 cm^2 sites on the ventral aspect of both forearms of 36 normal subjects. The sites were occluded with Saran Wrap® for the first 5 hours to increase absorption (i.e. make the rate of tretinoin absorption through forearm skin more like face skin). At each study visit, prior to dosing, a measurement of transepidermal water loss was taken with an electronic evaporimeter, and the sites were examined and graded for signs of desquamation (peeling/scaling).

0 no scaling/peeling
1 trace of scaling/peeling
2 up to 50% of the site peeled
3 >50% of the site peeled

The primary variables by which bioequivalence was determined were: (a) maximum transepidermal water loss achieved at any time during the study (TEWL), and (b) days-to-full-peel (DTFP, i.e. a grade of 3). Sites which had not peeled after days were assigned a value of 25 days for purposes of analysis.

Maximum TEWL

PLACEBO				
Product	N	Mean	SD	SE
SPEAR	68	0	0.4	0.1

DOSE = 0.025%				
Product	N	Mean	SD	SE
SPEAR 0.025%	34	12.3	11.9	2.0
RETIN-A 0.025%	34	12.1	12.1	2.1

DOSE = 0.01%				
Product	N	Mean	SD	SE
SPEAR 0.01%	34	7.8	8.6	1.5
RETIN-A 0.01%	34	8.2	9.6	1.6

Days to Full Peel

PLACEBO				
Product	N	Mean	SD	SE
SPEAR 0%	68	25*	0	0

DOSE = 0.025%				
Product	N	Mean	SD	SE
SPEAR 0.025%	34	18.0	5.1	0.9
RETIN-A 0.025%	34	18.1	5.3	0.9

DOSE = 0.01%				
Product	N	Mean	SD	SE
SPEAR 0.01%	34	21.7	4.6	0.8
RETIN-A 0.01%	34	22.1	4.9	0.8

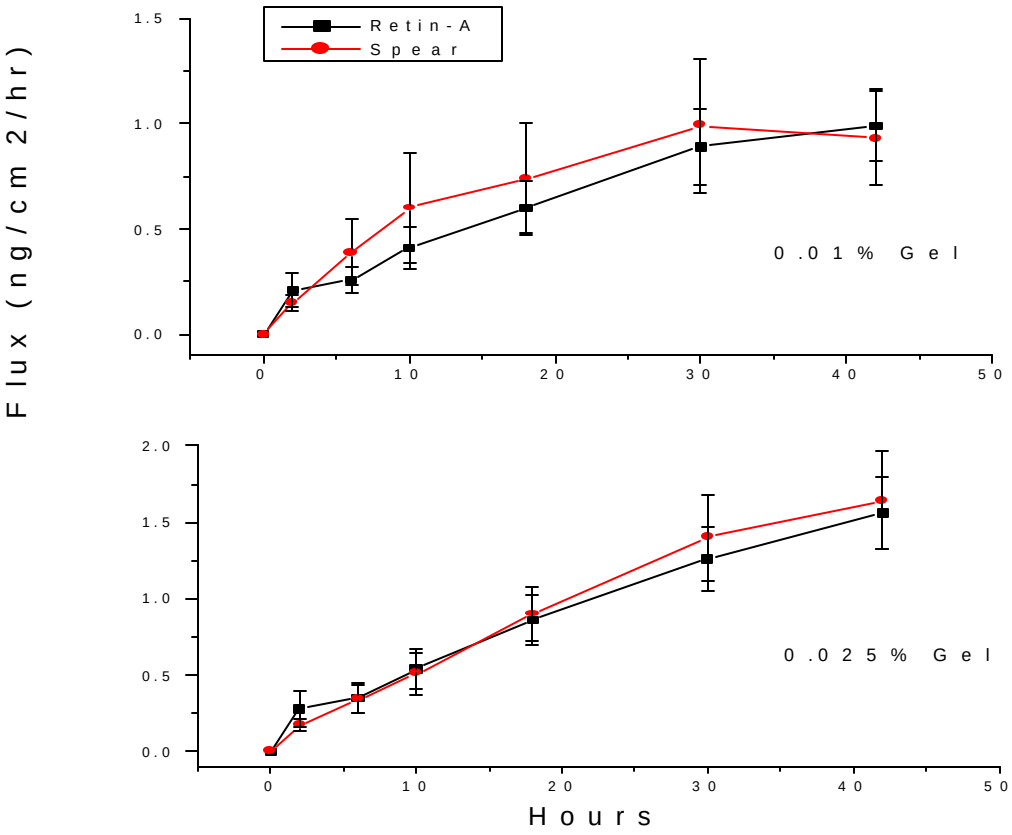
Summary of Statistical Analysis

Dose = 0.01%

TEWL: (93.9%, 114.7%)
DTFP: (95.8%, 103.5%)

Dose = 0.025%

TEWL: (97.8%, 121.03%)
DTFP: (96.3%, 105.2%)


Retin-A
Spear
0.01% Gel
0.025% Gel
Flux (ng/cm2/hr)
Hours
0.0
0.5
1.0
1.5
2.0
0
10
20
30
40
50

0.01% Tretinoin

Log Transformed Data

Parameter	Mean Spear	Mean Retin-A	Ratio (Spear/Retin-A)	90% Conf. Int. on Ratio
AUC	3.0752	3.0072	1.02266	97.07, 107.46
Fmax	0.6268	0.6173	1.03789	92.53, 115.05
Dermis	0.5920	0.7313	1.47803	108.24, 187.37
Epidermis	2.3357	2.3009	1.03373	97.90, 108.85
Surface	4.2454	4.2810	0.99239	98.05, 100.43
Total	4.4606	4.4794	0.99634	98.69, 100.58

Table 8
0.025% Tretinoin

Log Transformed Data				
Parameter	Mean Spear	Mean Retin-A	Ratio (Spear/Retin-A)	90% Conf. Int. on Ratio
AUC	3.4921	3.4709	1.02798	95.14, 110.45
Fmax	0.9058	0.8840	1.11481	95.08, 127.88
Dermis	0.6019	0.6334	1.36154	103.93, 168.38
Epidermis	2.2370	2.2401	1.01132	96.03, 106.23
Surface	4.2606	4.2762	0.99701	98.75, 100.65
Total	4.4325	4.4492	0.99675	98.88, 100.47